Three-Dimensional Desirability Spaces for Quality-by-Design-Based HPLC Development

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In this study, three-dimensional desirability spaces were introduced as a graphical representation method of design space. This was illustrated in the context of application of quality-by-design concepts on development of a stability indicating reversed-phase high-performance liquid chromatography method for the determination of vinpocetine and α-tocopheryl acetate in a capsule dosage form. A mechanistic retention model to optimize gradient time, initial organic solvent concentration and ternary solvent ratio was constructed for each compound from six experimental runs. Then, desirability function of each optimized criterion and subsequently the global desirability function were calculated throughout the knowledge space. The three-dimensional desirability spaces were plotted as zones exceeding a threshold value of desirability index in space defined by the three optimized method parameters. Probabilistic mapping of desirability index aided selection of design space within the potential desirability subspaces. Three-dimensional desirability spaces offered better visualization and potential design spaces for the method as a function of three method parameters with ability to assign priorities to this critical quality as compared with the corresponding resolution spaces.

Introduction

Quality-by-design (QbD) concepts have gained an increasing interest in many pharmaceutical development and manufacturing industries since the adoption of FDA and ICH to these concepts (1). Recently, the application of QbD concepts was extended to the development of analytical methods, especially reversed-phase high-performance liquid chromatography (RP-HPLC) methods (2). Methods developed according to the QbD-based approach could be characterized by robustness, ruggedness, selectivity and suitability for intended use as well as understanding of method parameter effects on method quality (2). In contrast with the traditional “trial and error” method development approach, the QbD-based method offered investment of time and effort of method development to define subspaces (rather than defined point) of method parameter combinations within the experimental domain satisfying the predefined method intents with the guarantee of method quality and robustness (3).

Many publications discussed the application of QbD principles to the development of RP-HPLC methods from different aspects such as presentation of work frames for QbD-based methodology (2, 4, 5), application of QbD elements for separation of certain mixtures (6–8) or innovation of methodology approaches for analytical QbD-based methodology (9–12).

Design space, an important element in QbD, was defined as the "multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality" (1). Conducting a method within the design space is not considered a change in method and, hence, does not necessitate method revalidation. The design space can be represented as a multidimensional model that relates multiple method parameters (e.g., pH, temperature or organic solvent for a chromatographic method) to method performance criteria (e.g., robustness, resolution or run time). Graphical representation of design space helps visualization and understanding of these multidimensional relationships (13).

Previous published works dealing with modeling and presentation of (potential) design spaces applied two-dimensional plots such as overlapping maps for multiple criteria (overlay plots), or desirability function plots to demonstrate the two-dimensional relationship between two of the method parameters with relevant method performance criteria (13). Many of these results were presented in two-dimensional plots for convenience, although the potential design spaces were obtained from multivariate optimization of three or more factors. Some of these multiple parameters were a mixture of continuous (e.g., pH and gradient time) with discrete parameters (e.g., column type or solvent type) (10).

Three-dimensional resolution spaces were introduced by Molnár et al. (11, 12) in the last versions of Drylab® software. Three-dimensional resolution spaces described graphically the effect of three different method parameters on predicted minimum resolution. This was in the form of either colored planes as segments within the three-dimensional space or alternatively by plotting zones exceeding resolution minimum limit in three-dimensional space in opaque. Several later publications supported the proposed value of the resolution spaces and the crucial role of visual representation of three-dimensional potential design spaces (14, 15). The three-dimensional tool was more convenient for potential design spaces representation and manipulation rather than handling of multiple two-dimensional planes. However, chromatographic separation optimization commonly involved other objective functions in addition to minimum chromatogram resolution (as minimum retention time or total run time). Desirability function could refine the resulting design spaces by restricting them to volumes complying with multiple criteria thus achieving multicriteria optimization. An additional advantage of using the desirability index optimization was the ability to optimize separations with assigning different priorities to each objective function to be optimized (13). Modeling uncertainty propagation into predicted responses is another important issue in the definition of design space borders for fulfillment of “assurance of quality” of the design space. Probability of a response to attain certain predefined values

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reflects the method quality on the basis of risk assessment (3). Potential design spaces, described only by average response values without consideration of their modeling uncertainty, do not provide assurance of quality.

The aim of the current work is to introduce a threedimensional desirability space tool as an alternative to three-dimensional resolution spaces for understanding of knowledge space and representation of potential design spaces. Three-dimensional desirability space is the plot of zones exceeding a threshold value of desirability function plotted in three-dimensional space defined by the axes representing three method parameters. It is important to note here the difference between the aforementioned desirability space and the three-dimensional desirability plot commonly described in the literature. The later actually reflects a two-dimensional relationship as only two axes were assigned for two method parameters while the third axis is assigned for the response value as a function of the two method parameters.

The probability of the predicted desirability index to attain certain values (thresholds) was calculated and used for assessment of resulting desirability spaces and selection of design spaces within them. The use of three-dimensional desirability spaces was demonstrated in the context of development of a stability-indicating RP-HPLC method for the determination of vinpocetine and \( \alpha \)-tocopheryl acetate in a capsule dosage form proposed for synergistic neuroprotective effects of both compounds.

Development of a single gradient chromatographic system for this particular mixture instead of two separate isocratic system for each component (which is a common situation in many laboratories) helps reducing the time and resources used for routine analysis of this product. Multicriteria optimization was expected to be demonstrated in this mixture as the multiple method performance criteria—total run time, minimum retention of early peaks of interest and minimum resolution would affect the method quality in different weights. In addition, this work was utilized in the context of verification of features and capabilities of software CHROMOCAD\textsuperscript{\textregistered} v.2, a homemade software developed in Microsoft\textsuperscript{\textregistered} Visual Basic 6 programming language (registration no. 821/2008 at Information Technology Industry Development Agency, Giza, Egypt). Microsoft Excel\textsuperscript{\textregistered} spreadsheets were used to confirm retention modeling and prediction obtained from the software.

MATLAB version 7.12.0.635 (R2011a), (MathWorks Inc.; Sherborn, MA, USA), was used to calculate and graph the dimensional plots and the three-dimensional desirability.

**Methods**

**Stress testing of the capsule dosage form**

The capsule contents were stressed at 70°C or at 70°C—75% relative humidity for 30 days following the procedure reported by Baertschi (16). Stressed capsule contents were extracted as described in the sample preparation procedure.

**Sample preparation procedure**

A quantity of powdered capsule contents equivalent to \( \sim 2.5 \) mg of vinpocetine and 37.5 mg of \( \alpha \)-tocopheryl acetate were extracted with 7 mL of a mixture of water and dimethylsulfoxide (2 : 5) with the aid of sonication. Then, the volume was completed to 50 mL with methanol–ethanol (1 : 1) and filtered. Five milliliters of the filtrate were diluted into 25 mL with methanol.

**Working standard solution**

Working reference materials of vinpocetine and \( \alpha \)-tocopheryl acetate were weighed and diluted with methanol–ethanol (1 : 1) to have a final concentration of \( \sim 10 \) \( \mu \)g mL\(^{-1} \) for vinpocetine and 160 \( \mu \)g mL\(^{-1} \) for \( \alpha \)-tocopheryl acetate.

**Forced degradation of vinpocetine and \( \alpha \)-tocopheryl acetate**

Individual solutions of either 0.5 mg mL\(^{-1} \) of \( \alpha \)-tocopheryl acetate or 0.25 mg mL\(^{-1} \) of vinpocetine in acetonitrile were degraded with 0.2 M hydrochloric acid and with 0.1 M sodium hydroxide at 70°C for 3 days. Then, they were neutralized and
completed to 50 mL with acetonitrile. Oxidative degradation was conducted in 3% hydrogen peroxide solution at the room temperature for 24 h.

Resolution solution preparation procedure
About 200 mg of α-tocopheryl acetate was refluxed with a mixture of ethanol:sulfuric acid:water (20:2.5:17.5, v/v/v) for 2 h. A volume of this solution containing 10 mg of total tocopherol was mixed with 1 mL of oxidized vinpocetine solution prepared by incubating 1 mg mL⁻¹ of vinpocetine solution in a mixture of acetonitrile and 30% H₂O₂ solution (3:1, v/v) at 60°C for 1 h, then mixed with 5 mL methanol and 2 mL triethylamine and completed to 25 mL with distilled water.

Preliminary experimental runs
To optimize the gradient time (Tg) and the acetonitrile blend ratio (R) in mobile phase organic component (B), a full factorial (2 × 3 levels) experimental design was performed. Two levels of Tg (18 and 6 min) and three levels of R (100% ethanol for R = 0, 100% acetonitrile for R = 1 and a mixture of acetonitrile—ethanol (1:1, v/v) for R = 0.5) were analyzed. All gradients started from %B = 70–100% and kept for 7 min at 100% before returning to 73%.

The forced degradation solutions, the stress tested dosage form of sample solution and the working standard solution were chromatographed in each of the six experimental systems. Retention times of vinpocetine and α-tocopheryl acetate peaks as well as their degradation product peaks were recorded for each experimental system and used for further modeling and prediction.

Modeling and prediction of identified peaks retention times
As only six experimental runs were required for modeling; peak identification and tracking process were conducted by separate injection of each individual compound as well as their degraded solutions under the six aforementioned experimental runs. In addition, dual-wavelength detection enabled tracking of different peaks by their spectral ratios between the two wavelengths. The retention times for identified peaks were modeled for simultaneous optimization of both gradient time and blend ratio.

The following LSST-based equation modeled retention under gradient elution mode (17):

\[
t_k = t_0 + t_d + \frac{100T_G}{\Delta B} \ln 10 \left( \frac{(t_C - t_0) \Delta B \ln 10}{100T_G} + 1 \right),
\]

where \(k_o\) is the capacity factor of the analyte under isocratic conditions of initial %\(B\), and \(S\) is a constant for each analyte. \(\Delta B\) is the difference in organic solvent concentration between gradient start and end, \(T_G\) is the gradient time, \(t_d\) is the system dwell time and \(t_0\) is the column void time.

Equation (2) modeled isocratic retention time as a function of ternary solvent ratio (18)

\[
\log k' = (p_1 + p_2 R + p_3 R^2) + (p_4 + p_5 R + p_6 R^2) %B/100
\]

where %B is the percentage of total organic solvents in the mobile phase and R is the ratio of volume fraction of the second organic solvent to B.

The procedure proposed by Heinisch et al. (19) was adopted for the prediction of retention times at any proposed gradient parameter elution and ternary solvent ratio. Calculation procedures for modeling and prediction of retention times were illustrated in the Supplementary data, Flow chart S1.

Three-dimensional desirability spaces generation
Method critical quality attributes (CQAs)—minimum resolution between critical peaks, retention time of last peak and retention times of first eluted peaks of interest (i.e., peaks to be determined or included in the resolution solution; in this case the peaks of vinpocetine and its oxidation product) were optimized simultaneously by the calculation of the global desirability index. Individual desirability functions were calculated for each predicted response to be maximized or minimized. To maximize an individual response, the individual desirability function \(d_i\) was calculated as

\[
d_i = \frac{\hat{y}_i - E}{F - E}, \quad \text{when } E < \hat{y}_i < F, \quad d_i = 0 \text{ when } \hat{y}_i \leq E \quad \text{and} \quad d_i = 1 \text{ when } \hat{y}_i \geq F.
\]

On the other hand, minimization of response could be calculated as

\[
d_i = \frac{F - \hat{y}_i}{F - E}, \quad \text{when } E < \hat{y}_i < F, \quad d_i = 1 \text{ when } \hat{y}_i \leq E \quad \text{and} \quad d_i = 0 \text{ when } \hat{y}_i \geq F.
\]

Although several formats of desirability function exist, the most simple is the linear form used in this study. Additionally, for factors having higher priority or weights, rising to power function makes the function nonlinear with higher slopes near the desired value (20).

\(E\) and \(F\) were the lower and upper limits, respectively, for the response \(i\) to be optimized and \(\hat{y}_i\) was the predicted value for response \(i\). Global desirability index D was then calculated from individual desirability values of all responses by the following equation:

\[
D = \left[ \prod_{i=1}^{n} d_i^{w_i} \right]^{1/n},
\]

where \(w_i\) through \(w_n\) are the priorities defined for each response for optimization. Increasing the value of \(w_i\) for a response \(i\) gives a nonlinear function that shifts selection toward the factors of given higher priorities.

Table I contains responses to be optimized, settings of partial desirability function used and priorities assigned for optimization of each response.

Successive two-dimensional matrices of global desirability as a function of both \(T_g\) and \(R\) were generated at different values of %\(B_0\) (64–82%). A three-dimensional desirability space was then generated using MATLAB® software by concatenating two-dimensional matrices representing the global desirability function calculations at different %\(B_0\) values into a single three-dimensional matrix, where the third dimension is the value of %\(B_0\).

The concatenation process applied did not interpolate intermediate values. However, this may happen upon three-dimensional plotting of isosurfaces in MATLAB®. To mitigate error in the
resulting plot, intervals between values of the third dimension were small enough (2% of $\%B$) to minimize the deviation from the interpolated values and predicted values.

The produced three-dimensional plots could be saved as MATLAB® figure files that may provide more practical manipulation of resulting three-dimensional spaces. Furthermore, these files could be transferred and manipulated by other computers apart from either modeling software or source modeling data used, thus facilitating method transfer. This could provide a vehicle for knowledge distribution about methodology without the need for expensive software packages in recipient laboratories.

Visualization of the potential design space was achieved by plotting of zones with the value of global desirability function exceeding a threshold value of 0.75 selected as design space border within the range from 4.70 to 19.60.

\[ \text{Table I} \]

<table>
<thead>
<tr>
<th>Response</th>
<th>Optimization</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>Priority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution between α-tocopheryl acetate and its related compound</td>
<td>Maximize</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Retention time of last peak (α-tocopheryl acetate)</td>
<td>Maximize</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Capacity factor of vinpocetine</td>
<td>Maximize</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Capacity factor of vinpocetine oxidative degradation product</td>
<td>Maximize</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

\[ \text{Table II} \]

<table>
<thead>
<tr>
<th>Exp. pH $T_R$ (min)</th>
<th>%RE</th>
<th>Blend ratio</th>
<th>$K_v$</th>
<th>$T_E$</th>
<th>$R_s$</th>
<th>$R_E$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.8</td>
<td>14</td>
<td>76</td>
<td>0.63</td>
<td>1.64</td>
<td>15.60</td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>14</td>
<td>76</td>
<td>0.57</td>
<td>1.52</td>
<td>15.98</td>
</tr>
<tr>
<td>3</td>
<td>5.4</td>
<td>12</td>
<td>76</td>
<td>0.63</td>
<td>1.96</td>
<td>14.86</td>
</tr>
<tr>
<td>4</td>
<td>5.4</td>
<td>12</td>
<td>70</td>
<td>0.57</td>
<td>1.58</td>
<td>14.97</td>
</tr>
<tr>
<td>5</td>
<td>5.8</td>
<td>12</td>
<td>70</td>
<td>0.63</td>
<td>2.15</td>
<td>15.37</td>
</tr>
<tr>
<td>6</td>
<td>5.8</td>
<td>14</td>
<td>70</td>
<td>0.57</td>
<td>2.08</td>
<td>16.47</td>
</tr>
<tr>
<td>7</td>
<td>5.4</td>
<td>14</td>
<td>70</td>
<td>0.63</td>
<td>2.07</td>
<td>17.10</td>
</tr>
<tr>
<td>8</td>
<td>5.8</td>
<td>12</td>
<td>76</td>
<td>0.57</td>
<td>1.53</td>
<td>14.33</td>
</tr>
</tbody>
</table>

\[ \text{Parameters effects} \]

\[ \text{Mean effects} \]

\[ \text{Margin of error (ME)} \]

\[ \text{K_v, capacity factor of vinpocetine peak; } t_R, \text{ retention time of α-tocopheryl acetate; } R_s \text{ and } R_E, \text{ minimum resolution of vinpocetine and α-tocopheryl acetate peaks, respectively.} \]

Uncertainty propagation to predicted retention data at any parameter combination was simulated by multiple random shifts to the predicted values according to $t$-distribution with the aforementioned standard deviation $S$ at the proposed conditions and degree of freedom equals to the number of confirmatory experiments.

This process was repeated ($n = 1,000$ times) for each predicted point, the desirability index was calculated from error propagated predicted retention times and the probability of desirability index to have certain values was calculated.

\[ \text{Robustness of the selected working method} \]

Eight experiments following the fractional factorial design of the order of $(2^{4-1})$ were used to study the effect of $%B$ pH of mobile phase buffer, $T_R$ and $K_v$ on method performance criteria. Selected method performance criteria were based on the resolution between vinpocetine and vinpocetine oxidative degradation peaks, the resolution between α-tocopheryl acetate and hydrolytic degradation (α-tocopherol) peaks, capacity factor $K'$ of vinpocetine and retention time of α-tocopheryl acetate. The design of robustness study is described in Table II. Dong’s algorithm was used to calculate the significance for chromatographic parameter effects on each of studied responses (21).

\[ \text{Method validation} \]

Linearity was performed by regression analysis of peak areas obtained from analysis of five different concentrations of the standard mixture ranged from 6.0 to 20.5 μg mL$^{-1}$ for vinpocetine and from 108.9 to 370.1 μg mL$^{-1}$ for α-tocopheryl acetate.

Repeatability was confirmed by evaluation of relative standard deviation of six individual determinations of vinpocetine and α-tocopheryl acetate. Intermediate precision was confirmed by one-way ANOVA evaluation of four independent assays at four different days.

Accuracy was determined by the analysis of nine sample solutions spiked with known concentrations of standard solution within the range from 4.70 to 19.60 μg mL$^{-1}$ for vinpocetine and from 92.01 to 368.03 μg mL$^{-1}$ for α-tocopheryl acetate followed by calculation of recovered amount of each compound in each spiked solution.
Selectivity was demonstrated by the resolution values between each of active ingredients and either their possible degradation products or possible foreign peaks from test preparation.

Method transfer
An initial isocratic hold of 2.0 min was added at the gradient program start in an experiment to simulate the effect of method transfer to a system with larger dwell volume on the method CQAs.

The stationary phase change effect was demonstrated by applying selected method conditions on two different C18 columns of brands different from the method column (Discovery HS C18 and Luna C18 (2)) as well as another aged column of the same package of the method column (Discovery C18).

Results
Optimized method conditions
A working point of the optimum desirability index was selected within the design space to be considered as the final method parameter. The selected method used the buffer, pH 5.6, as the aqueous component (A) and a blend of acetonitrile–ethanol (6:4, v/v) as the organic component (B) in the gradient program. Gradients began at 73% of the mobile phase organic component (B), increased to 100% in 13 min, held at 100% for 5 min, decreased to 73% in 1 min and finally held at this value for reequilibration for 4 min. The flow rate was 2 mL min⁻¹, and the detection wavelength was 275 nm.

Chromatograms obtained from analysis of resolution solution under the selected method conditions as well as chromatograms representing other degradation products were also presented in comparison with predicted chromatograms (Figure 1).

![Chromatograms of the final method conditions: (a) predicted, (b) resolution solution, (c) acid degradation of vinpocetine and (d) alkaline degradation of vinpocetine. Peaks captions: (i) vinpocetine, (ii) vinpocetine oxidation product, (iii) α-tocopherol, (iv) α-tocopheryl acetate, (v) α-tocopherol oxidation product, (vi) vinpocetine acid degradation product (apovincaminic acid) and (vii) vinpocetine alkaline degradation product.](https://example.com/chromatograms.png)

Rbias results for optimized conditions
The robustness testing results regarding changes in pH, T, R and initial %B₀ are presented in Table II with the significance of each method parameter on each tested response. According to Dong’s algorithm (21), all effects were below the margin of error (ME) value for each chromatographic response, indicating method robustness regarding these parameters.

Validation results
Repeatability showed relative standard deviation values of 1.38 and 1.85% for vinpocetine and α-tocopheryl acetate, respectively. Analysis of variance for inter-day assays exhibited F ratios of 0.99 and 3.53 for vinpocetine and α-tocopheryl acetate, respectively (F-critical = 3.86), indicating good intermediate precision.

The method was linear in the range of 6.02–20.48 μg mL⁻¹ for vinpocetine (r = 0.9998) and 108.86–370.12 μg mL⁻¹ for α-tocopheryl acetate (r = 0.9997). The detection limits estimated from calibration curves were 0.3848 and 10.2859 μg mL⁻¹ for vinpocetine and α-tocopheryl acetate, respectively. The quantitation limits were 1.16 and 30.89 μg mL⁻¹ for vinpocetine and α-tocopheryl acetate, respectively. The method accuracy was confirmed with average recoveries ± SD of 98.52 ± 0.56 and 99.16 ± 1.39% for vinpocetine and α-tocopheryl acetate, respectively, throughout the method linear range (Table III).

Method transfer
The effect of increasing dwell volume by up to 4 mL is illustrated in Table IV. Method CQAs did not fall beyond predefined thresholds upon dwell volume increment, indicating method transferability between instruments with different dwell volumes.

Method CQAs did not fall out of limits upon using an aged column of the same package or even using column packages of type B silica with hydrophobicity > 2.3 (approximate hydrophobicity of the method column) according to the Waters reversed-phase selectivity chart (Table IV).

![Table III Accuracy and Recovery Results for Vinpocetine and α-Tocopheryl Acetate](https://example.com/table.png)
Method parameters—gradient time, experimental design

The generation of three-dimensional desirability spaces for multi-experiments and predicted model evaluation and as a base for and targeted to be 1.5. These criteria were used further in initial.

The resolution of any active ingredient peaks if a stronger solvent (i.e., 100% ethanol) was used.

For sample preparation, ethanol–methanol mixtures ensured complete extraction of α-tocopheryl acetate because of its lower solubility in methanol. Methanol function was the reduction of solvent effects on the peak shape and location of early peaks if a stronger solvent (i.e., 100% ethanol) was used.

Required method performance criteria were defined according to the intended method scope; the determination of vinpocetine and α-tocopheryl acetate. The stringent approach described by Vogt and Kord (2) for the definition of the required method criteria was adopted. The resolution of any active ingredient peak should be not less than <1.5 and targeted to be 2, the retention time of the last peak should be not more than >30 min and targeted to be 10 min and the minimum capacity factor of the first eluted active ingredient peak should be not less than 1 and targeted to be 1.5. These criteria were used further in initial experiments and predicted model evaluation and as a base for the generation of three-dimensional desirability spaces for multi-criteria optimization of the method.

Choice of method parameters, model and experimental design

Method parameters—gradient time, $T_e$, and acetonitrile blend ratio, $R$, were selected to be screened for simultaneous optimization as they were expected to have the most probable influence on the method performance criteria. Other significant gradient parameters such as flow rate, initial and final gradient organic solvent ratios, $\%B_0$ and $\%B_{fin}$, were kept constant during the initial experiments but were optimized mathematically as the aforementioned LSST model was applied.

The buffer pH was kept constant at 5.6 to reduce ionization of the basic vinpocetine peak thus increasing its hydrophobicity and, hence, reduction of the distance between vinpocetine and α-tocopheryl acetate peaks and thus increasing vinpocetine.

Two main approaches were reported for modeling of chromatographic conditions: response surface modeling based on statistical design of experiments and the use of first principle equations (or mechanistic models) (13).

In gradient elution, many gradient parameters should be optimized: $T_e$, $\%B_0$, flow rate, $\%B_{fin}$, instrument dwell volume $V_d$ and column dimensions. For modeling of such many parameters, application of the first principle equations approach enabled the use of fewer initial experiments as compared with response surface modeling based on statistical experimental design (13). This approach enabled more extrapolation of prediction as well as taking into account factors such as instrument dwell volume that affects method transferability and thus method quality (17). Furthermore, equation (2) gave the best prediction results for ternary solvent ratio optimization (19).

Experimental runs evaluation and modeling of retention data

Peaks obtained from each run in initial experiments were identified as vinpocetine, vinpocetine acid degradation, vinpocetine alkaline degradation product, vinpocetine oxidative degradation, α-tocopheryl acetate, α-tocopherol and α-tocopherol oxidation product. The peak identity of α-tocopherol was confirmed by analysis of partially acid hydrolyzed α-tocopheryl acetate solution and in chromatograms obtained from stressed sample of pharmaceutical dosage form. Further neutralization of hydrolysis process liquor with exposure to air and light produced another peak which was suggested to be α-tocopherol oxidation product eluted in the midway between the two active ingredient peaks. Retention models were calculated for vinpocetine, its oxidative degradation product, α-tocopheryl acetate and α-tocopherol as they were observed in the initial experiments as the critical peak pairs impacting the assay of the two active compounds.
Table V
Experimental Conditions Used to Evaluate Prediction Models with Comparison between Predicted Retention Times \(t_{R \text{ pred.}}\) and Observed Retention Times \(t_{R \text{ obs.}}\) in Minutes for Vinpocetine, Vinpocetine Oxidation Product, \(\alpha\)-Tocopheryl Acetate and \(\alpha\)-Tocopherol

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Vinpocetine</th>
<th>Vinpocetine oxidation product</th>
<th>(\alpha)-Tocopheryl acetate</th>
<th>(\alpha)-Tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F_r ) (mL min(^{-1}))</td>
<td>(R)</td>
<td>(T_g ) (min)</td>
<td>(%B_0)</td>
<td>(%B_{fin})</td>
</tr>
<tr>
<td>a 1.5</td>
<td>0.25</td>
<td>6</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>b 1.5</td>
<td>0.25</td>
<td>5</td>
<td>70</td>
<td>95</td>
</tr>
<tr>
<td>c 2</td>
<td>0.50</td>
<td>10</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>d 2</td>
<td>0.75</td>
<td>4</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>e 2</td>
<td>0.75</td>
<td>9</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>f 2</td>
<td>0.75</td>
<td>4</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>g 2</td>
<td>1.00</td>
<td>10</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

\(F_r\), flow rate; \(R\), acetonitrile blend ratio; \(T_g\), gradient time (min); \(\%B_0\) and \(\%B_{fin}\), initial and final organic solvent percentages in gradient program, respectively; Diff, difference between observed and predicted retention times in minutes; \(S_{PE}\), prediction standard error.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Correlation between predicted and observed retention times for the modeled compounds.

To evaluate the calculated models, confirmatory experiments were done at conditions different from those used for model calculation. Estimated models had good prediction accuracy as illustrated in Table V. This was indicated by small differences in magnitude between the predicted and actual retention times (average of 0.22 min) and high correlation between the predicted and observed retention times for the four compounds \((R^2 = 0.996)\) (Figure 2).

**Three-dimensional desirability spaces generation and design space definition**

Method CQAs—minimum resolution between critical peaks, retention time of \(\alpha\)-tocopheryl acetate peak and retention times of vinpocetine and its oxidation product were used to calculate global desirability plots for multicriteria optimization as previously described (Table 1). Two-dimensional plots of global desirability as a function of gradient time \(T_g\) and blend ratio \(R\) at successive levels of initial \(\%B_0\) (with 3% interval in \(\%B_0\)) were demonstrated (Figure 3a–g), keeping the flow rate equal to 2.0 mL min\(^{-1}\) and final \(\%B_{fin}\) at 100% for time saving. It was clear that changes to \(\%B_0\) affected significantly the resulted desirability plots and subsequently, the method quality thus justifying the importance of \(\%B_0\) as a third parameter to model the knowledge space.

To achieve better visualization of potential design space and understanding of the method parameter effects on CQAs, three-dimensional desirability space was generated by plotting of zones having global desirability values \(>0.75\) as a function of \(T_g\), \(R\) and \(\%B_0\) (intervals of \(\%B_0 = 2\%\)) in three-dimensional space defined by \(T_g\), \(R\) and \(\%B_0\) as \(x\), \(y\) and \(z\) coordinates, respectively (Figure 4).

To compensate for model prediction uncertainty, boundaries of potential design spaces should be moved inside toward the optimum conditions to mitigate method failure risk at the margins (22). Therefore, threshold values of desirability space margins \((0.75)\) were selected although any non-zero value of global desirability function indicates that the method CQAs fulfill the minimum threshold of predefined method intents. This threshold value was justified by assuming that the threshold value \((0.75)\) should correspond to an individual desirability value of \(\sim 0.9\) for minimum resolution because of its highest priority \((value = 3)\), 0.87 for maximum retention time and 0.75 for minimum retention time. Consequently, we adjusted the threshold value of global desirability index to 0.75 to correspond to a resolution value of \(\sim 1.95\). This means that we can obtain methods satisfying most of intended attributes at the margins of potential design space.

**Desirability spaces in comparison with other plotting approaches**

Details of relationship of each individual CQA with the method parameters might be hidden within the represented domains of either desirability spaces or resolution spaces, especially when representing design spaces as zones exceeding a threshold value. Indeed, the use of individual plots for each response against one or two method variables is indispensable for understanding the role of variables–response relationship in method quality, which is a crucial aim of QbD-based approaches.

However, for definition of potential design space borders, desirability spaces may be more convenient than handling and visualization of multiple “two variables–one response” plots for each CQA at each level of the third optimized method parameter (in other words, too many plots would be produced from the
later technique in three-dimensional relationships). Instead, one plot of desirability spaces could substitute many plots of “two variables–one response” in the task of potential design space borders definition. Furthermore, different weights (priorities) could be assigned for each optimized criterion by using desirability function. This will give an extra advantage over individual response plots that the represented optimized domains would consider the relative importance of each optimized variable within the method, analytical target profile. This weighing ability of global desirability index could not be determined visually from individual response plots. In addition, the optimization between responses to be maximized and responses to be minimized cannot be visualized on multiple plots of individual responses but could be simply plotted with desirability functions.

Figure 5 exemplifies the comparison between the conventional two variables ($T_G$ and $R$)–one response individual plots and their corresponding desirability function plot at $\%B_0 = 73\%$ (plane including working conditions). It was obvious that the desirability function plot (Figure 5c) described optimum zones and thus border of potential design space in a more convenient way than using individual CQAs plots. It was clear that for optimization in the third dimension ($\%B_0$), many plots would be needed corresponding to the individual desirability plots (Figure 3a–g) or the single desirability spaces plot (Figure 5).

To compare three-dimensional desirability spaces with three-dimensional resolution spaces, the later were generated by plotting of volumes having a minimum resolution value of $\sim 1.95$ (corresponding to a resolution desirability value of 0.75 with

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**Figure 3.** Global desirability function plots against $T_G$ and $R$ at different $\%B_0$ values: (a) $\%B_0 = 64\%$, (b) $\%B_0 = 67\%$, (c) $\%B_0 = 70\%$, (d) $\%B_0 = 73\%$, (e) $\%B_0 = 76\%$, (f) $\%B_0 = 79\%$ and (g) $\%B_0 = 82\%$.

**Figure 4.** A three-dimensional desirability space for design space representation illustrating zones in space with a desirability function of $>0.75$ against $T_G$, $R$ and $\%B_0$ from two different views.
weight = 3) as a function of \( T_G \), \( R \) and \( \%B_0 \) in three-dimensional space (Figure 6). In comparison with the corresponding three-dimensional desirability spaces (Figure 4), resolution spaces classified larger volumes as suitable design space although a portion of these volumes were not suitable for method operation regarding other CQAs. This could be observed at higher values of gradient time \( T_G \), intermediate \( R \) values and extreme values of \( \%B_0 \). On the other hand, the use of desirability index had the advantages of multicriteria optimization of several CQAs, weighing and assigning priorities of these criteria. The three-dimensional desirability spaces, therefore, provided a more reliable way to represent potential design space by the aforementioned virtues inherited by the use of desirability function.

**Probabilistic evaluation of desirability spaces and design space definition**

From the inspection of the resulted three-dimensional desirability spaces, two distinct volumes of high global desirability values were observed. One of them located at low \( R (<0.1) \) and low \( \%B_0 \)
(<70%) values and the other located at intermediate \( R \approx 0.6 \) and higher \( \% B_0 \) (70–80%) values.

Although the later volume appeared larger than the other to justify its selection as a potential design spaces, such selection should be based on assessment of modeling uncertainty propagation, thus fulfilling the QbD key requirement of risk assessment (3).

The probability of the predicted global desirability index to attain the proposed threshold value (0.75) was calculated throughout the knowledge space (Figure 7a). Higher probability values were obtained at intermediate \( R \) values and \( \% B_0 \) values between 70 and 76%. A more detailed two-dimensional \( (T_G, R) \) probability plotting was calculated with overlaying the cross-sectioned border of resulting desirability spaces (bold thick borderline) at \( \% B_0 \) values of either 73 and 66% (Figure 8a1 and a2, respectively). Desirability spaces at intermediate \( R \) values and higher \( \% B_0 \) (Figure 8a1) enclosed higher probabilities to pass the defined desirability threshold than those at lower \( R \) values and lower \( \% B_0 \) (Figure 8a2).

Additionally, the same decision could be concluded by plotting the probability for the method to satisfy the minima of the predefined objectives (i.e., less risk of failure) as the probability of global desirability index to exceed zero. Such probability was plotted in three dimensions (Figure 7b) and in two-dimensional \( (T_G, R) \) probability plotting with overlaying the cross-sectioned border of resulting desirability spaces at \( \% B_0 \) values of either 73 and 66% (Figure 8b1 and b2, respectively).

According to the aforementioned risk assessment, the desirability subspace located at \( R \) values of \( \approx 0.6 \) and \( \% B_0 \) at 70–76% was selected for design space definition.

According to ICH Q8 (1), design space could be defined as a combination of proven acceptable ranges of parameters \( T_G, \% B_0 \) and \( R \). The design space was defined as a cube based on selected desirability subspace and its probabilistic evaluation as well as robustness results. Defined cube had the dimensions of gradient time \( T_G \) from 11 to 14 min, acetonitrile blend ratio \( R \) 0.5–0.7 and initial \( \% B_0 \) from 70 to 76%.

**Control strategy definition**

Based on method transfer results and robustness worst-case analysis, two control strategy elements were defined. The first was the control of stationary phase type to be restricted to silica type B-based C18 packages with hydrophobicity not less than 2.3 according to the Waters reversed-phase selectivity chart. The second was the system suitability test limits for resolution between vinpocetine and its oxidative degradation product to be not less than 4.5, the capacity factor of vinpocetine peak to be not less than 1.7, the retention time of \( \alpha \)-tocopheryl acetate to be not more than 18 and the resolution between \( \alpha \)-tocopheryl acetate and \( \alpha \)-tocopherol to be not less than 2.

**Conclusion**

Optimization of gradient parameters—gradient time, initial and final organic component percentages and flow rate as well as

![Figure 7](https://academic.oup.com/chromsci/article-abstract/53/4/467/345370/Downloaded-by-guest-on-12-March-2019)

Figure 7. Three-dimensional plots of: (a) probability \( P > 0.65 \) to attain global desirability threshold of 0.75 and (b) probability \( P > 0.8 \) to attain global desirability values >0.

![Figure 8](https://academic.oup.com/chromsci/article-abstract/53/4/467/345370/Downloaded-by-guest-on-12-March-2019)

Figure 8. Contour plots \( (T_G, R) \) of (a1, a2) probability \( P > 0.65 \) to attain a global desirability threshold value of 0.75 and (b1, b2) probability \( P > 0.8 \) to attain global desirability values >0, at two levels of \( \% B_0 \): (a1, b1) \( \% B_0 = 73\% \) and (a2, b2) \( \% B_0 = 66\% \). The bold line indicated intersected desirability space border. The circles in (a1, b1) indicated working point.
ternary solvent ratio were optimized with only six initial experiments because of the merit of using retention models based on the first principle equations without impacting model prediction power. Three-dimensional desirability spaces illustrated potential design subspaces as a function of gradient time, blend ratio and initial organic solvent percentage. Three-dimensional desirability spaces provided better representation for the borders of potential design subspaces which were based on multicriteria optimization than other conventional representation methods. The probabilistic study of resulted subspaces helped risk-based selection of the proper design space. The utility of the three-dimensional desirability space tool should be assessed for other future mixtures to explore its practical reliability and applicability.

**Supplementary data**

Supplementary data are available at *Journal of Chromatographic Science* online.

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